Short Communication

Considerations for post-lethality treatments to reduce *Listeria monocytogenes* from fully cooked bologna using ambient and pressurized steam

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Abstract

During processing of ready-to-eat (RTE) deli meats, any secondary processing procedures such as peeling and cutting introduce the distinct possibility of cross-contamination between equipment, personnel, and food. To eliminate or reduce pathogens such as *Listeria monocytogenes* and ensure food safety, RTE deli meats can be pasteurized prior to or after packaging. In this study, ambient steam in-package pasteurization was compared with pressurized steam prepackaging pasteurization to reduce *L. monocytogenes* from fully cooked RTE bologna. The bologna (14 cm diameter × 1.5 cm thickness) samples were surface-inoculated to contain about $8 \log_{10}$ of *L. monocytogenes*. To achieve 2 log reductions for *L. monocytogenes*, the bologna samples needed to be treated for about 10 s in pressurized steam at 131 °C or for about 2.5 min in ambient steam at 100 °C. The pasteurization time using pressurized steam treatment was about 75–90% shorter than using ambient steam treatment. Pressurized steam treatment may be integrated into a vacuum packaging unit to effectively eradicate *L. monocytogenes* from RTE meats just prior to sealing the retail packages to further reduce the treatment time, avoid post-treatment recontaminations by pathogens, and improve food safety without detrimentally affecting meat quality.

1. Introduction

Many of today's concerns for pathogens such as *Campylobacter jejuni*, *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Cyclospora cayetanensis* were not recognized as causes of foodborne illnesses about 20 years ago (Mead et al., 2000). Foodborne illness bears a high cost to the US economy and results in thousands of deaths each year. According to the estimate by the Centers for Disease Control and Prevention, foodborne diseases cause approximately 76 million illnesses, 325,000 hospitalizations, and 5000 deaths in the United States each year, in which about 14 million illnesses, 60,000 hospitalizations, and 1800 deaths are caused by known pathogens (Mead et al., 2000).

Three pathogens, *Salmonella*, *L. monocytogenes*, and *Toxoplasma*, are responsible for 1500 deaths in US each year with *L. monocytogenes* responsible for about 500 deaths among 2500 illnesses (Mead et al., 2000). About 99% of listerosis cases reported in US were transmitted by foods (USDA-FSIS, 2003a). *L. monocytogenes* accounts for 28% food-related death and the case-fatality of listerosis was 20–30%—the second highest among bacterial diseases (Mead et al., 2000).

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L. monocytogenes is a pathogenic bacterium found in soil, water, and vegetation and on the surface of equipment, floor, and walls and is often carried by healthy animals as well as humans (USDA-FSIS, 2003a).

L. monocytogenes easily spreads by direct food contact with a contaminated surface, possesses a relatively high resistance to heat and salt concentration, and can grow at refrigeration temperatures as low as $2\,^\circ C$ or under low oxygen tension such as found in vacuum-packaged RTE meats (USDA-FSIS, 2003a; Samelis et al., 2002). While the cooking processes currently applied by the US meat and poultry industry generally meet United States Department of Agriculture Food Safety and Inspection Service (USDA-FSIS) requirements, the processing steps after cooking such as peeling, sorting, loading, slicing, packaging, etc., are potential sources of recontaminations for pathogens such as *L. monocytogenes*. A USDA-FSIS survey published in 2001 showed that 1–10% of retail RTE meat and poultry products was contaminated with *L. monocytogenes* (Levine et al., 2001).

Illness outbreaks associated with *L. monocytogenes* in RTE meats have prompted governmental regulatory agencies to impose a new rule on October 6, 2003 for *L. monocytogenes* reductions (USDA-FSIS, 2003b). This new rule requires that all establishments that produce RTE meat and poultry products that are exposed to the environment after lethality treatments will need to develop written programs, such as Hazard Analysis and Critical Control Point (HACCP) systems, Sanitation Standard Operating Procedures (Sanitation SOPs), and other prerequisite programs, to control *L. monocytogenes* (USDA-FSIS, 2003b).

While various methods have been used to control recontaminations from *L. monocytogenes*, each method has some key disadvantages. For example, one option for controlling *L. monocytogenes* is to handle products in aseptic environments. Although sanitation policies have helped to improve sanitary conditions in RTE meat and poultry processing plants, true aseptic conditions are extremely difficult to achieve (Levine et al., 2001; Tompkin, 2002). *L. monocytogenes* cannot be completely eliminated from RTE meat and poultry processing environment using present technologies (Berrang et al., 2002; Tompkin, 2002). The potential contaminations of *L. monocytogenes* on RTE meat and poultry products presents a food safety threat and has promoted interest in applying post-cook pasteurization treatment prior to or after packaging to reduce *L. monocytogenes* incidences among RTE meats.

Post-cook pasteurization by steam or hot water was used to reduce *Salmonella* or *Listeria* from RTE meat and poultry products (Gill et al., 2001; Kozempel et al., 2000; Muriana et al., 2002; Murphy et al., 2001a; Murphy and Berrang, 2002a, b; Murphy et al., 2002a–c,

2003a–d). Considering large volumes of varieties of RTE meats on the market today, the industry is in need of information that could help RTE meat and poultry processors choose a simple and cost-effective post-lethality treatment alternative that is feasible to their processes to improve food safety without negatively affecting quality of their products.

The objective of this study was to compare the effectiveness of in-package ambient steam treatment with prepackaging pressurized steam treatment as commercial alternatives on reducing *L. monocytogenes* from fully cooked bologna. Although high temperature short time (HTST) treatment has been applied in treating liquid foods such as fruit juice, pressurized steam pasteurization has not been commercially used in treating RTE meats due to the difficulties of commercial implementation when using a stand-alone pressurized steam cooker for RTE meat products and re-exposure issues after pasteurization treatment prior to retail packaging. From this study, we propose to apply prepackage pressurized steam pasteurization technology in each of individual vacuum packaging chambers of a packaging machine to reduce *L. monocytogenes* from RTE meat products and minimize the re-exposure time of the treated meats to processing environment before final retail packaging of RTE meats is completed.

2. Material and methods

2.1. Product

Fully cooked bologna logs (14 cm diameter × 30 cm length) were obtained in plastic packages (0.2 mm thickness) from a processor. The bologna samples were analysed to contain about 28% fat, 15% protein, 5% carbohydrate, 1% sodium salt, and 50% moisture. The ingredients of the bologna was consisted of pork, mechanically separated chicken, dextrose, corn syrup, salt, beef, water, flavorings, granulated onion, and granulated garlic. The formulations of the bologna were proprietary to the processor. The packaged bologna logs were kept at $-20\,^\circ C$ and thawed at $4\,^\circ C$ for about 24 h prior to each use.

2.2. Culture preparation

Six strains of *L. monocytogenes* (ARS#V105, ARS#V67, ARS#V72, ARS#V113, ARS#V125, and LCDC 81-861 4b) were obtained on the slants containing tryptic soy agar (TSA) plus 0.6% yeast extract (YE) from M.E. Berrang at USDA-ARS-RRC (Athens, GA) and M.G. Johnson at the University of Arkansas (Fayetteville, AR). From each slant, a loopful of each culture was transferred to 10 ml tryptic soy broth (TSB) plus 0.6% YE in a test tube and then incubated at $35\,^\circ C$

for 24 h as stock cultures. One ml of each stock culture was transferred to 9 ml of TSB + 0.6% YE culture and incubated at 35 °C for 24 h as substock cultures.

Each tube of the substock cultures was vortexed and 1 ml of each substock culture was transferred to a 250 ml flask containing 100 ml of sterile TSB + 0.6% YE. The culture flasks were incubated on an orbital shaker at 600 rpm and 35 °C for 24 h. Each culture was enumerated to contain 10^9 cell forming units (CFU) per ml of culture medium. Just prior to inoculating bologna samples, an equal volume of each culture was pipetted into a sterile flask and mixed to obtain a cocktail culture.

2.3. Surface inoculation

Each bologna log (4 °C) was carefully unpackaged and sliced by a meat slicer (Model 300, Globe Slicing Machine Company Inc., Stamford, CT) into 1.5 cm thick × 14 cm diameter sections. The slicer was cleaned with soap water, water, and then 75% ethanol between each use. In a laminar flow hood, each bologna sample was placed in a sterile pan containing 50 ml of the *L. monocytogenes* cocktail culture and slowly rotated to allow the culture to cover the entire surface of the bologna samples. The surface-inoculated bologna samples were placed on a sterile rack for 5 min to allow excess fluid to drip off.

Considering that the surface temperature of the bologna samples might increase during inoculation handling, after surface inoculation, the inoculated bologna samples were kept in a refrigerator at 4 °C for 60 min to allow the surface temperature of the inoculated bologna to equilibrate (determined by preliminary tests using a Type E surface thermocouple probe, Omega Engineers, Stamford, CT). In each test trial, the inoculated and unheated bologna samples prepared according to the same procedure as above were used as controls to calculate the initial CFU of *L. monocytogenes* on bologna samples.

2.4. Heat treatment

Some of the inoculated bologna samples were individually vacuum-packaged (Komet, Stuttgart-W, Kornbergstr 27–29, Germany) in 0.2 mm thick packaging films (Tyson Foods Inc., Springdale, AR) and then heat-treated in an ambient steam cooker (60 cm × 60 m × 120 cm, FMC, Sandusky, OH) at 100 °C for different periods of time from 0 to 8 min. After heat-treatments, each sample was immediately submersed in an ice-water bath (0 °C) to cool. The other inoculated bologna samples were individually heat-treated in a pressurized steam cooker (20 cm × 25 cm × 25 cm, G. Dwyer, Winslow, AR) at 131 °C for different periods of time from 0 to 2 min.

After heat-treatments, each pasteurized bologna sample was immediately placed in a sterile plastic bag, vacuum-packed in 0.08 mm thick packaging films, and submersed in an ice-water bath (0 °C) to cool. During treatments, meat temperatures were monitored via a data acquisition unit (23 × Omega Engineers, Stamford, CT) using Type E (0.2 mm diameter, Omega Engineers, Stamford, CT) thermocouple probes inserted at different locations of a meat sample. The heat transfer coefficient and velocity were determined according to the procedures similar to the descriptions by Murphy et al. (2001b).

2.5. Microbial enumeration

After cooled for about 60 min, the bags of bologna samples were blotted with paper towel and wiped with 75% ethanol. Each bag was carefully cut open using a sterile blade. Three hundred ml of sterile 0.1% peptone solution was poured into each bag. The bologna sample in the bag was massaged by hands for 5 min to make sure that all surfaces of the bologna sample were in contact with the peptone solution. Then, serial dilutions were made in sterile 0.1% peptone solution and spread-plated in duplicate onto thin-layer-agar plates that consisted of Modified Oxford medium (MOX) overlaid with 5 ml of TSA + YE (0.6%) to resuscitate heat-injured cells (Kang and Fung, 1999).

Our preliminary study showed no significant differences on the CFU counts for the recovered heat-injured cells between an incubation temperature of 35 and 25 °C except it took a longer period of time for the cells to appear on the plates at 25 °C than at 35 °C. Therefore, an incubation temperature of 35 °C was used in all test trails. The viable colonies were counted after incubating the plates at 35 °C for 48 h and the plates were kept at 35 °C for a total of 5 days during which the plates were examined daily for additional colonies. To detect low levels of *L. monocytogenes*, an enrichment procedure was used. Twenty-five millilitres of the initial bologna/peptone rinse solution were added to 225 ml of TSB + 0.6% YE and incubated at 35 °C overnight followed by the USDA-FSIS method to detect *L. monocytogenes* (Johnson, 1998).

2.6. Data analysis

A total of about 790 inoculated bologna samples were tested in this study with an initial inoculation level of about 10^8 CFU/cm^2 for *L. monocytogenes*. The products were surface-inoculated with *L. monocytogenes* prior to each pasteurization treatment using ambient or pressurized steam. Entire product surfaces were analysed for the pathogen survivors. The amount of *L. monocytogenes* remaining on the bologna was enumerated after pasteurization and expressed as CFU/cm^2 of product

surface area. The total product surface area was about $374\,cm^2$. Three bologna samples were treated at each treatment time and used as one datum point. The goal of the analysis was to determine if there were significant differences in *L. monocytogenes* survivors between ambient and pressurized steam treatment over time. A negative binomial model was performed to analyze the data using SAS version 8.1 (copyright 1999–2000, SAS Institute Inc., Cary, North Carolina).

3. Results and discussion

Fig. 1 shows the $\log_{10}(CFU/cm^2)$ reduction of *L. monocytogenes* from bologna samples that were treated by ambient steam at $100\,°C$ or pressurized steam at $131\,°C$ (40 psi). The symbols were experimental data and the lines were model predictions within 95% confidence limits. For an initial inoculation level of $10^8\,CFU/cm^2$, no survivors of *L. monocytogenes* were found on bologna samples after the inoculated bologna were treated in ambient steam for 8 min or in pressurized steam for 2 min. No physical (texture and colour) differences were obtained between the untreated bologna samples and the samples treated for up to 8 min in ambient steam or for up to 2 min in pressurized steam (data not shown).

In a separate study, the changes of physical properties and sensory characteristics were evaluated using packaged RTE chicken strips (454 g/package) treated in ambient steam or hot water at $88\,°C$ for 34 min, targeting at achieving at least $7\log_{10}$ reductions of *L. monocytogenes*. There were no significant (at $\alpha = 0.05$) differences found on instrumental texture (shear force) and sensory attributes (springiness, cohesiveness, hardness, and chewiness) between treated and untreated

RTE chicken strips (data not shown). However, additional water purge was detected in the packaged RTE chicken strips after treating the chicken strips at $88\,°C$ for 34 min in ambient steam or hot water (Murphy and Berrang, 2002a).

Additional water purge occurred in the RTE meat packages during in-package pasteurization treatment may have a negative effect toward consumers' perception on product quality. Therefore, besides in-package pasteurization, the meat and poultry industry also needs an alternative that is simple, fast, easily implemented, and cost-effective to handle various types of RTE deli meats in large volumes.

For prepackaging pasteurization under pressurized steam, because a short-time heat treatment was applied prior to final retail packaging, the water purge problems relating to heat-treating meat or poultry products in packages is less an issue of concern. However, due to potential re-exposure of the treated meats to processing environment, there is a risk of recontaminations by pathogens after prepackaging pasteurization treatments. Therefore, it becomes critical to apply prepackaging pasteurization treatment at the point close to final retail packaging.

When commercially applying pressurized steam treatment on to prepackaged RTE meat products, pressurized steam can be introduced into each individual vacuum packaging chambers of a packaging machine to form a steam-pasteurization/vacuum-packaging-in-one unit, allowing the meat products to be vacuum-packaged immediately after the post-lethality treatment to eliminate any potential pathogen recontaminations prior to retail-packaging of RTE meat products. Introducing pressurized steam into space-restrained and tightly controlled vacuum-packaging chambers also further reduces pasteurization time by increasing steam velocity on meat surfaces and
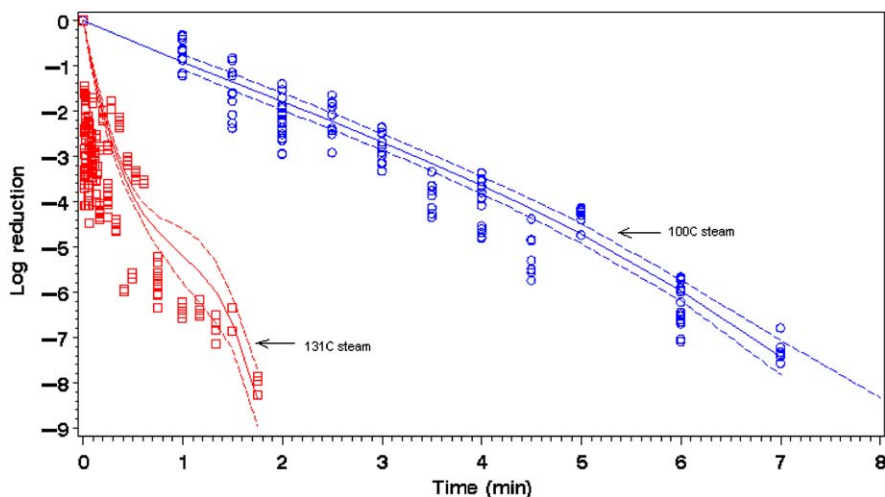

Fig. 1. Log reduction of *L. monocytogenes* in fully cooked bologna (14 cm diameter × 1.5 cm thickness) pasteurized by ambient steam at $100\,°C$ or pressurized steam at $131\,°C$. Symbols were experimental data and the lines were model predictions with 95% confidence limits (dashed lines).

enhances the treatment repeatability by minimizing the variations on heat transfer coefficients between heating medium and meat surfaces.

The time needed for post-lethality treatment of RTE meat products was affected by cooker designs such as heat transfer coefficient and circulation velocity. In our study, for ambient steam pasteurization, the average heat transfer coefficient was $758 \, W/m^2 \, K$ and the steam velocity was about $0.5 \, m/s$. Increasing heat transfer coefficient and steam velocity will increase the heating rate and subsequently reduce the heat-treatment time. The pressurized steam pasteurization from this study had an average heat transfer coefficient of about $4011 \, W/m^2 \, K$ and a steam velocity of about $5 \, m/s$, resulting in a much shorter pasteurization time than that using ambient steam treatment.

When applying in-package pasteurization, a thicker packaging film has to be used for retail packages of RTE meat products. Thicker (0.2–0.28 mm) packaging films not only have a higher (50%) cost than normal thickness (0.08–0.15 mm) of the films but also create a barrier to heat transfer. Increasing packaging film thickness slowed heat penetration rate through the packaging and subsequently reduced the thermal inactivation rate of pathogens in packaged meat samples (Murphy et al., 2002a).

The pasteurization time using pressurized steam treatment was 75–93% shorter than using ambient steam treatment. From this study, to achieve 2 logs of reduction for *L. monocytogenes*, the bologna samples (14 cm diameter × 1.5 cm thickness) needed to be treated for about 10 s in pressurized steam at 131 °C, comparing to 2.5 min in ambient steam at 100 °C for the same log reductions. Pressurized steam provided a higher steam temperature than ambient steam and therefore using pressurized steam was able to significantly reduce pasteurization time. Kozempel et al. (2000) conducted surface pasteurization study using pressurized steam at 138 °C by alternatively applying multiple cycles of steam and vacuum on hotdogs and obtained 3–5 $\log_{10}$ reductions of *L. innocua* in less than 3 s of a total operation time.

In a previous study using ambient steam pasteurization at 85–95 °C for 4–8 min, Gill et al. (2001) achieved 4–5 $\log_{10}$ reduction of *L. monocytogenes* on RTE meat products including beef salamia and turkey Kielbasa. Muriana et al. (2002) found that *L. monocytogenes* inoculated on RTE turkey, beef, and ham were reduced 2–4 logs after heat treatment in 90–96 °C water bath for 2–10 min. Using fully cooked and vacuum-packaged chicken breast meat products, Murphy and Berrang (2002a, b) evaluated post-lethality treatment using steam and hot water and found that both treatments were equally effective in reducing *Salmonella* or *L. innouca* at the same temperature (88 °C). According to Alkar-RapidPak (Lodi, WI), the equipment cost of an ambient steam pasteurizer is about the same as an ambient hot water pasteurizer.

Integrating pressurized steam into individual vacuum-packaging chambers of a packaging machine for RTE meats would also substantially increase the economical returns to the users. As shown in Table 1, comparing with an ambient steam pasteurizer of similar production capacity, pasteurization/packaging-in-one unit could reduce the investment cost by about 50%. By applying pressurized steam of about 130 °C in a vacuum-packaging system for 1.5–2 s, about 2.5 $\log_{10}$ reductions of *L. monocytogenes* were obtained for double-layer packed (8 links/package) hotdogs and about 4 $\log_{10}$ reductions of *L. monocytogenes* were obtained for single-layer packed (6 links/packages) hotdogs (unpublished data, Alkar-RapidPak, Lodi, Wisconsin, 2002).

Because the roughness on meat surfaces provides harborage for pathogen cells to hide, pasteurization time needed for achieving certain pathogen reductions on meat is also affected by crevices, dents, cuts, folds, netting marks, cracks, wrinkles, or tears present on meat surfaces. Based on a previous study, some crevices on turkey breast meat products were as deep as 35 mm (Murphy et al., 2003d). For in-package heat pasteurization, to eliminate the hidden pathogen cells in deep crevices, dents, cuts, folds, netting marks, cracks,

Table 1

Process economical analysis comparing ambient steam pasteurization with a pasteurization/packaging-in-one unit. (based on single-layer packed hotdogs in 6 links/package)

Parameters	Ambient steam pasteurizer[a]	Pasteurization/packaging-in-one unit[a]
Capacity	3000 kg/h	3500/h
Floor print	12 m length × 2 m width	9 m length × 1.5 m width
Targeted $\log_{10}$ reduction	4 logs for *L. monocytogenes*	4 logs for *L. moncytogenes*
Effect on product quality and process	Generate drips in the packages	Not affect product quality in preliminary tests by Alkar-RapidPak
	Need a specific packaging film (8–11 mils)	Not need for a specific packaging film
Processing time	Minutes	Seconds
Installation cost	$250,000[b]	Replace the existing packaging equipment
Equipment cost	$600,000–$800,000	$340,000 per unit

[a] By Alkar-RapidPak, Lodi, Wisconsin.
[b] In most cases, factories need to be extended to include the new pasteurizing equipment at a cost of $2000–$2700 m^{-2}.

wrinkles, or tears, heat needs to penetrate through a certain depth of the RTE meat or poultry product via conductive heating.

Since many RTE meat and poultry products are ground, formed, and cooked in a casing, the roughness on a meat product surface is irregular and inevitable, which complicates the process validation for pathogen reductions on these products when applying an in-package heat pasteurization treatment. The pasteurization time needed for each individual meat product must include the time necessary for heat to reach the deepest crevice, dent, cut, fold, netting mark, crack, wrinkle, or tear that may be contaminated with pathogen cells via diffusion through aqueous phase on the product. For a RTE turkey breast product (9 lb formed) with a crevice of about 15 mm deep, it could take 50 min of in-package heat pasteurization time to reduce 7 logs of *L. monocytogenes* in the crevice (Murphy et al., 2003d).

During pressurized steam treatment, high pressure steam momentarily expanded the crevices, dents, cuts, or tears on RTE meat products, allowing high velocity of steam in direct contact with pathogen cells in these crevices, dents, cuts, netting marks, wrinkles, or tears on meat. Thus, pressurized steam treatment is more effective in eradicating pathogens on meat than using in-package heat pasteurization. Introducing pressurized steam into each individual vacuum packaging chambers of a packaging machine reduces void spaces between heating medium and meat products, shortens the contact time between steam and meat, and subsequently decreases treatment time. Therefore, the best place to apply pressurized steam is inside vacuum-packaging chambers of an RTE meat packaging machine at the step right before sealing the top films of the meat packages.

Once a meat product is pasteurized, substantial cooling time may also be needed to cool the treated meat product to 4 °C. The cooling time needed after in-package ambient steam treatment was much longer than that of prepackaging pressurized steam treatment due to a greater penetration depth into the meat during in-package ambient steam treatment. At 0 °C, about 31 min of cooling time was needed to cool an in-package heat-treated turkey breast product (9 lb packages) from 71.1 to 3.9 °C (Murphy et al., 2003d). In a pasteurization/packaging-in-one unit, immediately applying vacuum following short-time pressurized steam treatment also helps to dissipate heat from the treated meat surfaces.

4. Conclusions

A comparative study was conducted for *L. monocytogenes* reduction from fully cooked bologna that was surface-pasteurized via ambient steam at 100 °C or pressurized steam at 131 °C. Comparing with ambient steam treatment, using pressurized steam to treat RTE meat products substantially reduces the pasteurization time and improves meat quality. However, pressurized steam treatment should be applied at a point immediately before vacuum-sealing the top films of the retail packages of RTE meats to avoid any potential recontaminations of pathogens on the treated meat products. This study may help the RTE meat and poultry producers to choose different alternatives of post-lethality treatments to reduce potential contaminations of pathogens from RTE meats so that to improve food safety and avoid costly recalls.

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